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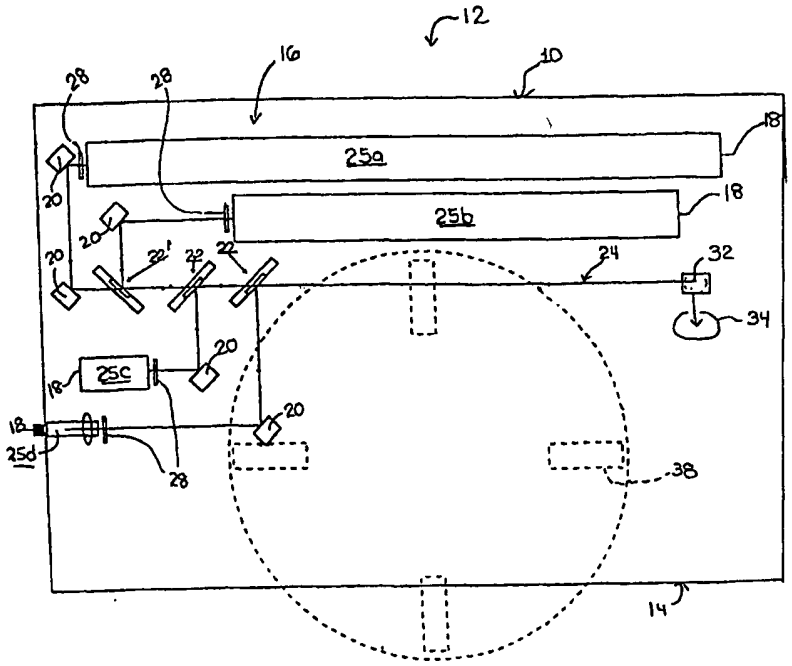

(57) Abstract

A laser-based optical scanning system (12) includes a laser delivery system (16), a laser-based scanning subsystem (36), a laser-based detection subsystem (44), and an automated stage (54) for processing multiple slides (38). The laser-based scanning subsystem (36) includes a galvanometer mirror (32) for scanning a laser beam along the X-direction. The automated stage (54) includes a linear stage for scanning the beam along the Y-direction.

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LASER-BASED OPTICAL SCANNING SYSTEM

FIELD OF THE INVENTION

The present invention generally relates to a laser-based optical scanning system. More particularly, the present invention relates to an automated, laser-based optical scanning system suitable for efficiently and reliably scanning a plurality of microscope slides in a laboratory or research environment.

BACKGROUND OF THE INVENTION

Functional genomic research, especially biochip imaging, has become extremely competitive. In an effort to keep up with increasing demands, researchers continue to seek faster, more efficient and reliable data acquisition equipment. Laser-based scanning systems are a type of system that has been developed to advance research and improve productivity. Moreover, such imaging systems are commonly used to capture imaging information from fluorescently labeled materials, such as cDNA spotted on microscope slides.

Unfortunately, most currently available imaging systems are stage-scanning type instruments that only accommodate one slide at a time. Over time, the large amount of travel needed to scan a whole slide at a reasonable speed introduces wear and tear, which can result in alignment problems, breakdowns and unscheduled down time. Other systems that are available typically employ a telecentric scan lens, which require lateral chromatic and monochromatic corrections and adjustments, limiting their wavelength range and making them expensive.

Current laser scanning systems collect excitation laser light that is reflected from the slide surface along with fluorescence down the emission beam path. This light is typically rejected with multi-layer emission filters, however, even at 10^{10}

blocking, the emission filters let through a small amount of light that appears as background in the image. The present invention employs a dark field technique that blocks the slide reflection before it reaches the emission filters, reducing background and thus increasing sensitivity.

Based upon the aforementioned limitations, there exists a need in the industry for an automated laser-based scanning system that can efficiently collect the desired imaging data in a more efficient and reliable manner while reducing expected maintenance and/or repair or replacement and reducing background with a dark field technique.

SUMMARY OF THE INVENTION

Recognizing the limitations associated with currently available laser-based imaging systems, the present invention provides a laser-based scanning instrument particularly suited for automated laser-based imaging applications. A particular example of such an application is the scanning of fluorescently-labeled cDNA that has been spotted on a microscope slide by a robot. The present invention utilizes a unique scanning method that allows for high fluorescence collection efficiency through simple on-axis optics, while maintaining the improved-speed advantages of scanning the laser beam with a galvanometer mirror. The present invention also includes the application of confocal techniques, which offer improved resolution and improved rejection of out-of-focus noise, or alternatively provides a large depth of field thereby permitting the incorporation of automation schemes for imaging multiple slides. The present invention also includes opto-mechanical features, which permit dark-field use, increasing the overall sensitivity of the invention.

A principal feature of the present invention is its ability to provide an automated system that is capable of efficiently accommodating a plurality of slides. The stage/galvo scanning embodiment disclosed in connection with the present invention permits researchers to scan at much faster rates than is possible with currently available stage-scanning instruments. Further, because the present invention employs a galvanometer mirror, or oscillating mirror, to scan one axis, the system is able to address a broader wavelength range, i.e., into the ultraviolet (UV) and near-

infra red (IR) ranges, while maintaining a high numerical aperture for signal collection.

Because the resolving power is determined by the numerical aperture of the incident laser beam, a narrow beam produces a larger spot on the sample and, in addition, makes the depth of field deeper. The on-axis optics of the present invention provide a significant advantage over other laser-based scanners in that no lateral chromatic or monochromatic corrections are required, reducing the expense of the system and reducing the design constraints associated with use of a wider range of wavelengths. Moreover, the large depth-of-field permits simple adaptation to a wider range of slide automation equipment than systems that are currently available from conventional scanners.

An additional advantage of the system is that it can be optimized for improved-speed scans of typical robot-spotted cDNA samples. Scanning resolution, i.e., pixel size, can be optimized to help prevent under-sampling of cDNA spots and avoid possibly missing strands of labeled cDNA. The system is also capable of working with multiple dyes and provides for the use of multiple detector types that can address the sensitivity and dynamic range requirements of the samples. Because of the on-axis optics involved, the technique is very adaptable to the introduction of many types of lasers and power levels, including those not within the visual range. Given sufficient laser power, a single strand of cDNA can be imaged by the system.

A laser-based scanning system is described, which is suitable for improved-speed automated imaging of multiple slides in a laboratory, clinical, diagnostic or research environment. The laser-based optical scanning system includes a laser delivery system, a laser-based scanning and detection system, and an automated stage for sample handling. The optical design includes at least two mirrors, at least one dichroic or Rugate mirror for combining excitation and emission paths, and may include least one penta prism mirror combination for imparting mechanical stability through the scanning mechanism. One embodiment scans the objective lens in one direction and uses a galvanometer driven mirror for the second axis. An alternative embodiment scans the objective in two directions, using two scanning stages. In both cases, the light path may be diverted with a pentaprism to maintain perpendicular

alignment during a scan. An automated stage transports a plurality of slides past the laser-based scanning and detection system.

In a preferred embodiment, the scanning system includes at least two base plates -- a first, or upper base plate, and a second, or lower plate positioned below the upper base plate. The use of multiple plates creates multiple levels. A first level is mounted on top of the upper plate and includes a laser delivery system. A second level is preferably mounted underneath the upper plate and includes laser-based scanning and detection components. A third level is mounted on the lower base plate and includes components for an automated stage for processing multiple slides. The third level is controllably positioned an appropriate distance from the first level so that the slide on the third level is located at the focal point of the scanned laser beam. The laser beam originates on the first level and travels to the second level through a hole in the upper plate, thereby separating most of the laser illumination scatter from the collection area of the second level. A dichroic mirror or Rugate filter directs the laser beam through the optical path to the slide positioned above the lower plate, where the beam can excite fluorescence from the sample. The fluorescence is collected along the same optical path, but passes through the excitation dichroic or Rugate filter, and continues to the fluorescence detection system along the emission path. A galvanometer mirror scans the laser beam along the X-direction, i.e., the "fast axis," while a linear stage scans the laser beam along the Y-direction, i.e., the "slow axis."

BRIEF DESCRIPTIONS OF THE DRAWINGS

The features and inventive aspects of the present invention will become more apparent upon reading the following detailed description, claims, and drawings, of which the following is a brief description:

FIG. 1 is a top plan view of the level one components of a preferred embodiment of the present invention as seen looking down upon an upper base plate thereof.

FIG. 2a is a top plan view of the level two components of a preferred embodiment of the present invention as seen looking through the upper base plate.

FIG. 2b is an illustration showing two mirrors, the penta prism and the affect of adjustment on beam deflection.

FIG. 3 is a top plan view of the level three components of a preferred embodiment of the present invention as seen looking down upon a lower base plate.

FIG. 4a is an illustration of a side view of the slide scanning equipment.

FIG. 4b is an illustration of the mirror with respect to the slide and doublets and various swing angles of the mirror with respect thereto.

FIGS. 5a and **5b** illustrate the interrelationship between mirror size and objective size.

FIG. 6 illustrates a top view of a preferred emissions path.

FIG. 7 illustrates a top view of an alternative preferred emissions path to that illustrated in **FIG. 6**.

FIG. 8 is a top view of a preferred embodiment of the system.

FIG. 9 illustrates an objective scanner.

FIGS. 10a and **10b** illustrate several optical techniques associated with an objective scanner.

FIGS. 11-14 illustrate opto-mechanical features which permit operation as a dark-field instrument and increase the overall sensitivity of the invention.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

A preferred embodiment of the present invention is hereinafter disclosed in terms of plates, or multiple "levels." However, it should be understood that the invention is not limited to a device or system having a particular number of levels. In fact, if desired, all of the components of the present invention can be included on a single level.

FIG. 1 shows a top plan view of level one 10 of an optical laser-based scanning system 12. The first level 10 is positioned above the upper base plate 14 and includes a laser delivery system 16. The laser delivery system 16 is generally comprised of a plurality of lasers 18 of various types, including HeNe, solid state,

fiber-delivered, or other conventional types. In a preferred embodiment, corner mirrors 20 and dichroic mirrors 22 are used to combine the beams selected from up to four or more lasers into a common laser beam 24. However, under normal sequential imaging only one of the lasers is on at a time. Simultaneous imaging could be accomplished by opening shutters to 2 or more lasers. The embodiment depicted in **FIG. 1** shows four different lasers: a red He-Ne laser 25a, a yellow He-Ne laser 25b, a green solid state laser 25c, and a Blue Argon fiber-delivered laser 25d.

The dichroic mirrors 22 are generally color-selective mirrors that reflect a particular band of spectral energy while transmitting others. In a preferred embodiment, all of the dichroic mirrors 22 are adjustable and reflect the shorter wavelength yet pass longer wavelengths. Each individual beam may be deflected by two adjustable mirrors, a dichroic 22 and a mirror 20, or other types of conventional beam modifiers, to better adjust the co-alignment of the beams.

In a preferred embodiment, each laser 18 has at least one dedicated shutter 28 to control the passage of the associated beam. The shutters 28 permit the user to select and control the combination and intensity of beams that are brought together into a combined beam 24. There is also a safety shutter which prevents laser light from exiting the upper level. Computer software and/or central processing units can be used to more effectively control the lasers 18, the shutters 28, and/or mirrors 22. A neutral density filter (not shown) can be added to the individual laser paths or to a common path of more than one combined beam. If the laser beam power does not saturate the fluorophore, the lasers 18 can be used at full strength to maximize the fluorescence output and signal-to-noise of the data collected.

A reflecting mirror 32 may be used to deflect or conduct the combined beam 24 through an aperture 34 in the upper base plate 14. The combined beam 24 is then directed to the scanning subsystem 36 by way of excitation dichroic 42. (Fig. 2a). The scanning subsystem 36 is preferably positioned below the upper plate 14 and, more preferably, may be connected or secured to the underside of the upper plate 14. The aperture 34 separates most of the laser illumination scatter from reaching the fluorescence collection area on the second level. The reflecting mirror 32 directs the combined beam 24 through the optical path to the slide 38 (represented by

hidden lines in this figure). The majority of conventional slides 38 are rectangular in shape and have what is referred to as a short, "X" axis and a long, "Y" axis. The combined beam 24 is used to excite fluorescence in the sample 40, and the fluorescence given off from the sample 40 is collected along the same optical path as the beam 24. However, the fluorescence emitted from the sample 40 passes through the excitation dichroic 42 and proceeds to a fluorescent detection system 44 along the emission path.

FIG. 2a is a top plan view of the level two 46 of a preferred embodiment of the scanning system 12 as seen looking through the upper base plate 14 with the level one 10 components removed. Preferably, this second level 46 includes an excitation dichroic or single Rugate filter 42, a scanning subsystem 36, and emission path with a detection subsystem 44. The detection subsystem 44 may additionally include fluorescence detection optics and detectors.

As previously described, the combined beam 24 passes from level one 10 to level two 46 through an aperture 34, preferably in the upper base plate 14. The excitation dichroic or Rugate 42 reflects laser light from the combined beam 24 towards the scanning subsystem 36. Fluorescence returning from the scanning components 36 is conducted through the dichroic 42 to a fluorescence detection subsystem 44. If desired, a dichroic slider 50 containing multiple dichroics 42 can be used to optimally select different laser beams from the combined beam 24 and pass the longer-wavelength fluorescence. A fluorohore is a group of atoms that give a molecule fluorescent properties. Because dichroic mirrors have the capability of reflecting a plurality of laser lines, while passing fluorescent emissions, it is possible to collect data from a single fluorophore or multiple fluorophore simultaneously. A dichroic mirror that reflects only one laser line can be better optimized for fluorescence throughput, and single-channel sequential imaging minimizes cross-talk between fluorophores. Conversely, multi-channel simultaneous imaging reduces the amount of time required to scan a sample. In an alternately preferred embodiment, a multiline Rugate filter can be used to reflect at least four laser lines, while efficiently transmitting the emitted fluorescence in multiple bands, thereby eliminating the need to co-align multiple dichroics in the dichroic slider 50. While such Rugate filters

transmit fluorescence more efficiently than dichroics, they are typically more expensive, but avoid the labor complexity associated with co-aligned dichroic sets.

A beam expander 52 expands the laser beam 24a (see Fig. 2a) to allow for appropriate diffraction-limited resolution when the beam 24a is focused. The expander 52 also reduces the size of the returning fluorescent beam 24b, so smaller filters can be used in the emission path.

The optical scanning system 12 includes a stage 54 (see Fig. 2a) that moves two mirrors and an on-axis objective lens in the longer, "Y-direction" to scan the laser beam 24 along the long or Y-axis of the slide 38. Preferably, the stage 54 is designed to minimize the effects of vibration. For example, to minimize the vibration and jitter of the stage 54 in the Y-direction, the optical hardware can be mounted on two cross roller positioning stages. Such cross roller positioning stages offer exceptional reliability, wear, and low friction with minimal backlash or side play.

In a preferred embodiment, the top and bottom mounting surfaces of the stage 54 are precision machined to provide micro-flat mounting surfaces and the stage drive mechanism (not shown) incorporates a precision ground anti-backlash lead screw assembly. The radial loading design of the ball lead screw eliminates high drag torque and has inherent dampening qualities for applications requiring vibration control. A zero backlash flexible coupling can be used to connect a micro stepper drive motor and the ball lead screw assembly. A one-piece construction can often be used to eliminate mechanical joints and potential failure points. In addition to being torsionally rigid, requiring no lubrication, such a construction also accommodates large angular and axial misalignment. Preferably, all machined parts are made from what is commonly known as aluminum tool and jig plate -- which is dimensionally stable and readily available. The flexibility in component design for the stage 54 and associated components allows for maximum Y-direction stage 54 stability, and ease of both assembly and testing, which provides a highly reliable modular design with low maintenance.

The motor used in connection with the stage 54 is preferably a stepping motor 55. Software and hardware can be developed for the motor to allow microstepping, which results in smoother movement and operation of the stage 54. However, other

motors can also be used. For example, a DC servomotor with a high resolution and complex position feedback system can be employed, however, in order to provide smooth, constant velocity motion, the servo system must be carefully tuned to the mechanical drivetrain. Likewise, one could employ a galvanometer motor, driving the stage via pulley and metal band; however, this requires very fine control of an analog position signal as well as careful tuning. A coupler between the motor and drivetrain can help minimize vibration and to transmit forces more uniformly (independently of motor angle), so as not to cause periodic motion irregularities.

The combined laser beam 24 is directed to a galvanometer mirror 56 that scans the beam along the short, X-axis of the slide 38. The galvanometer mirror 56 provides fast, variable, but accurate scanning of the laser beam in the X-direction, and the stage 54 may be programmed for use at different speeds and step sizes for variable and accurate scanning in the slow, Y-axis direction. This approach permits faster scanning than is usually possible with a two-axis stage/sample-scanning system.

The fluorescent light emitted from the sample 40 on the slide 38 is collected on a large scan mirror 58 and is descanned. Because mirrors have zero chromatic aberration, the descanned beam 60 remains stationary. The descanned beam 60 is collimated by the achromatic objective 68 and travels toward the photo multiplier tubes (PMTs).

In a preferred embodiment, two mirrors 64 are configured like a pentaprism to minimize scan jitter, and simplify alignment. As illustrated in **FIG. 2b**, small rotations around depicted axis A or B (wherein axis B is perpendicular to the page) results in almost no change in the right angle or 90-degree deflection of the laser beam. This feature allows particularly simple mounting of this optic. Small rotations around depicted axis C or D give a 1:1 deflection in the laser beam, whereas the deflection would double with a single mirror. This feature helps minimize jitter from stage motor vibration in the images. Alternatively, these two mirrors could be replaced by a corner mirror 66.

The objective power is selected and/or adjusted to focus the laser beam 24a on to the sample 40 positioned on a slide 38. Although the laser beam 24a is narrow, the objective, pentaprism-like mirrors 64 and scan mirror 58 are comparatively larger in

size to help collect the largest possible beam 24b of emitted fluorescent light from the sample 40. The configuration of a preferred embodiment, such as that shown in **FIG. 2a**, focuses the beam 24 to about a 20-micron spot (width at the 1/e points), allowing the scanner to resolve 13 micron point objects or smaller. In such a scanning configuration, the optical resolution is determined by the laser spot size, not the detector. In this embodiment, the objective 68 is made achromatic, although this is not necessarily required.

FIG. 3 is a top plane view of a level three 76 (Fig. 4a) and associated components of a preferred embodiment of the present invention as seen looking down upon a lower base plate 78. The level three components preferably include an automated handling stage 54 (see Fig. 2a), and more particularly, a computer-controlled rotary stage 80. However, the rotary stage 80 shown can be replaced by an X/Y stage, a linear belt automation system, a slide auto-loader, or the like. Virtually any slide-loading apparatus with an appropriate depth-of-field tolerance, typically about 1mm or less, can be used. The primary slide loading position is generally designated by LP.

As generally depicted, a protective shield 82 can be incorporated into the system 12 to protect both the user from laser light and the PMTs from natural or room light. If desired, a bar-code reader 84 can be mounted above or in close proximity to the stage 80, to identify slides 38 as they pass by, or slides 38 can be read with a hand-held bar-code reader (not shown) before being placed in the unit or, alternatively, after exiting the system 12.

FIG. 4a is a side view of a portion of the system 12 that further describes and illustrates the primary slide scanning components. The height H of the scan mirror 58 and optical path above the sample, the objective focal length, and the laser beam width can all be balanced to optimize the smallest diffraction-limited laser spot, largest depth of field, and minimal field curve. The slide 38 is positioned at the objective focus. The height of the optics centerline above the slide 38 is determined by the distance to the focal point of the objective 68. For example, a 60mm mirror 58 will typically yield a numerical aperture of approximately 0.3. Achromatic doublets 86, such as those made of standard glass, can be used in connection with an aperture

88 so that the right side of the field will be the same in brightness as the left side of the field. Without the aperture, the right side will be up to 30% brighter. As a practical matter, some distance D (for example, about 18 mm.) is needed to fit a cover above the stage and below the lens. Further, relatively large collection apertures, such as 90 and 92, allow high fluorescence collection efficiency. In a preferred embodiment, the lens or lens set has a focal length of about 129 mm and is about 100 mm wide to catch all of the rays and have the laser beam on center. Return rays cover 78 mm, so pentaprism mirrors have to be about 78 mm long, up and down. In connection with this arrangement, it is kept in mind that a smaller laser beam provides for a larger depth of field.

Scanning the focused laser beam through an arc causes a field curve. If the distance from the scan mirror to the sample is too short, the field curve becomes too large, and the laser spot will go out of focus and increase in size at the edges of the sample. This scanning method allows for high resolution (for example about 13 microns or more), high NA (about 0.3), and improved-speed imaging (in excess of 4,000,000 points in less than one minute), without requiring a complicated and expensive imaging scan lens. It is easily adaptable to any wavelength range, because the objective 68 is used on-axis, and no lateral color correction is required. The large depth of field (e.g., 1mm) allows this scanning method to be easily adapted to a wide range of automation schemes for scanning multiple slides. The large depth of field also allows for scanning through conventional coverslips.

In accordance with a preferred embodiment, the pixel spacing is generally determined by the scan angle and stage stepping. As shown in **FIG. 4b**, the mirror 58 will have a normal scan angle that is ± about 5 degrees mechanical, plus overscan. A clearance zone distance CZ is provided for safety in case of excessive mirror swing. The pixel spacing is software controlled and can vary from 1 um to over 100 um. The optimal pixel size is ½ of the width of laser spot at the l/e points, or smaller (less than or equal to 10 microns). This pixel spacing ensures no strands of DNA are missed from undersampling the DNA spot with the laser beam.

FIGS. 5a and **5b** illustrate the relationship between mirror size and objective size. **FIG. 5a** depicts a configuration in which the mirror 58 is oversized and the

objective size is minimized. **FIG. 5b** depicts a configuration in which the mirror size is minimized, the objective is oversized and the lens is off-center. It is important to note that both methods can provide a numerical aperture of about 0.35 with an evenly illuminated field.

With an oversized mirror 58 and minimized objective size, such as shown in **FIG. 5a**, the user can achieve an even field of illumination by using the lens 90 on-center with an aperture and an oversized scan mirror that is positioned off-center. In such a configuration, the scan mirror area is designated as 92 and the scan mirror 58 should be substantially symmetrical for the galvo to rotate properly. In a preferred embodiment, the mirror 58 is about 128 mm wide and the numerical aperture is approximately 0.365. Further, cone angles may vary from about 39.9 in the middle to about 38.9 at the edge, the brightness difference being about 5%.

Using an oversized off-center objective lens 90, such as illustrated in **FIG. 5b**, allows for reduction of the scan mirror size while maintaining a similar numerical aperture (NA), and keeping the field illumination generally even. The surface area of the mirror that is used is smaller (e.g., 60 mm versus 128 mm) because a larger portion of the mirror 58, such as that below the optical axis, is used for light collection. The rays in this region are more concentrated so that more light is collected on that side of the mirror. The rays above the optical axis have a greater distance to diverge and are therefore less concentrated. In such a configuration, the minimum lens size is preferably about 92 mm (3.7 in.) to catch all of the rays and have the laser beam on-center. Line **L** indicates the point at which the lens 90 may be cut off, if desired. Preferably, the return rays cover about 67mm, so pentaprism-type mirrors have to be about 67mm long, up and down. Further, an aperture is generally needed or the right side of the field will be about 30% brighter than the left side of the field. Such an aperture compensates for numerical aperture differences and evens out the field of illumination. In the example shown in **FIG. 5b**, the measured cone angles are: 35.3 (Left), 38.2 (Right), and 40.3 (Middle), with no aperture, yielding a field variation of about 30%.

The reduced scan mirror size allows for the use of a cheaper, lower-powered galvo to drive the mirror. The aperture corrects the field variation caused by the small

variation in the cone angle that the scan mirror accepts, as the scan angle changes. This numerical aperture variation allows more light to be collected on one side of the field of view than the other. The objective is much wider than required to focus the narrow laser beam. Using the narrow laser beam with a large collection aperture allows optimization of the depth of field for automation and cover-slipped samples, while maintaining a high NA to maximize fluorescence collection efficiency.

If desired, a focusing lens and confocal aperture, such as those represented in **FIG. 6**, can be added to the system 12 to reduce the depth of field, thereby minimizing collection of fluorescence from contaminants on the back of the slide. The aperture 88 can also minimize background fluorescence from out-of-view particles lit up by scattered laser light. The aperture 88 would not be required with carefully handled slides, non-fluorescent opaque slides, or with slides that are painted on the back with a non-fluorescent paint before they are processed. The focusing lens 90 could be used without an aperture to reduce filter sizes and the required PMT aperture size such as shown in **FIG. 7**. Further, charge-coupled device (CCD) pixels could be used as a confocal aperture.

With regard to the detector 94, multiple PMTs allow for simultaneous imaging of two or more fluorescence colors. While three PMTs are shown in the embodiment depicted in **FIG. 6**, i.e., a logarithmic PMT 98, a true integration PMT 99, and a photon counting PMT 100, more could be added. Single strands of DNA may give out 100 photons or less, making a photon counting channel appropriate for lowest level signals. Photon counting is a digital technique that is the most sensitive alternative with the best signal to noise ratio, but it has a limited dynamic range, is best for dim samples, and is the most complex. There is no pixel-to-pixel smearing with photon counting. True integration electronics developed for a PMT give the most accurate signal (other than photon counting) but with a larger dynamic range than photon counting. A true charge integrator is an analog system that gives the best S/N ratio for analog systems, and can have multiple dynamic ranges by switching the integration capacitor (or integration times), and retains the zero crosstalk (smearing) between pixels. It must be followed by a traditional A/D converter. A traditional low pass filter is the most simple design, but has more noise, has pixel-to-pixel smearing, is not easy to range switch, and must also be followed by a traditional A/D converter.

PMTs are capable of producing a larger dynamic range than other detectors. Logarithmic A/D electronics for the PMT could give a maximum dynamic range, while maintaining sensitivity for low-level message cDNA signals. The dynamic range of expressed proteins in a cell can exceed 500,000:1, requiring a 19 bit dynamic range. The logarithmic PMT 98 could compress this dynamic range into smaller image file sizes. To handle large dynamic ranges in the same sample (without doing some kind of range switching), a log amp can be used at the front end of the analog electronics. This log function can be used in either the integrator or the low pass filter designs, but is not normally used in photon counting. It trades dynamic range for resolution by presenting the log of the PMT output to the integrator or low pass filters. These designs tend to be complicated, somewhat noisier, and have drift problems. Other detectors could also be appropriate for this application. For high signals, such as fluorescence obtained from labeling all of the cDNA spotted down on the slide as a control, a silicon diode detector may be appropriate, such as illustrated in **FIG. 7**. A mini-CCD detector, for instance a 16x16-pixel array, would work well for this application, and would be able to be binned and/or read out at high speeds. A mini CCD detector would have up to 5 times the quantum efficiency of a PMT detector, and could also increase the dynamic range of the system with binning. The PMT gain can be adjusted to increase the photon signal above the inherent noise of the PMT and readout electronics. CCD's do not have this gain feature, limiting their signal to noise. However at low light signals, Schott noise is the limiting noise factor, which is dependent on the number of collected photons. The increased quantum efficiency of a typical low-noise CCD (~7 electrons noise) still allows a signal-to-noise increase of about a factor of two over a PMT, because of the increased number of photons collected. Either one or a small number of CCD pixels could be used as a confocal aperture, such as shown in connection with **FIG. 7**.

The beam expander 52 reduces the beam of emitted fluorescence (emission beam) to a smaller size, allowing the use of smaller filters. The fluorescence travels through the excitation dichroic to an emission dichroic wheel, which directs the fluorescence colors to appropriate fluorescence filter wheels and PMTs. The filters can be positioned on the wheels or sliders. Stationary dichroic filters 104 can be used in place of the emission dichroic filter wheels, as depicted in **FIG. 7**. The optical

scanning method causes the emission beam to be parallel, allowing for the most stringent filter designs possible, which can reject the laser excitation light by a factor of 8 dB or more. Adding a focusing lens to the path causes the beam to converge, and places more difficult design constraints on the filters. However, high-rejection filters can handle some angle variation, and this can be taken into consideration in the overall design. Maximum rejection of the laser excitation is required so that the laser light does not contribute to background when detecting the smallest fluorescence signals from single cDNA strands. With this highly adaptable design, a wide variety of fluorophores can be accommodated in the same instrument.

FIG. 8 is a composite view of a preferred embodiment of the system. This particular figure illustrates the three layers 10, 46, 76 of scanner components overlaid upon one another and viewed from above.

FIG. 9 is an illustration of an objective scanning option that can be used in connection with the present invention. A second scanner embodiment is one that scans a much smaller objective in both X and Y directions by using a vertical pentaprism 108 for scan stability. This embodiment of the present invention provides for a much higher collection efficiency, and higher spatial resolution than the galvanometer scanner, however, in practice it may be slower. This embodiment also provides a large depth of field, and lends itself to automated slide handling.

In the instant embodiment, the objective can be scanned in both X and Y directions, using pentaprisms to aid in alignment stability and vibration minimization. The fast scan stage scans a small objective and vertical pentaprism in the X direction. The fast scan could cover 25 mm or more, the width of a slide, or could cover only the width of a patch of DNA spots, to aid in stability and speed of scanning. An X-stage, holding the slide, could move the next row of patches or the next slide into view of the scanner. This sample stage could also act as a multiple-slide loader.

The on-axis layout of the scanner allows for use of a very small, lightweight objective with a very high NA (>0.75). The high NA of the objective allows for maximal light collection efficiency, and allows for a higher resolution scan, depending on the choice of the laser beam width. The laser beam width and focal length of the objective will determine the diffraction-limited laser spot size at the

sample, which will in turn determine the resolution of the image. The depth of field also decreases with a decrease in laser spot size, and so these two requirements must be balanced in the final system. Diffractive, refractive, gradient index (grin) lenses, or combination lenses could be used for the objective. The objective would need to be well enough corrected for on-axis monochromatic errors to allow the resolution desired for the unit, with the chosen laser beam diameter. With a very short focal length (8 mm or less) objective, the returning emitted fluorescence beam will be quite small, and the pentaprisms can also be quite small and light, allowing faster scanning.

FIGS. 10a and **10b** depict additional optical techniques associated with an objective scanner in connection with the present invention. **FIG. 10a** demonstrates how a laser beam condenser (reverse beam expander) 110 can be used to reduce the laser beam width to an appropriate size to achieve the desired depth of field. The depth of field becomes larger as the laser beam width becomes narrower at the objective 112. Individual beam condensers 110 could be placed in each laser beam path, to independently focus the laser beams and correct partially for chromatic error in the objective 112. As shown in **FIG. 10b**, the preferred embodiment would be to use a somewhat longer focal length objective, and focus the shorter-wavelength laser 114 short of the objective's back-focus 118. The objective would need to be optically corrected for use at this focal point. The sample would be placed at this focal plane. The longer-wavelength laser beams 116 would be focused more strongly as they impinge upon the objective, to correct for the color errors in the objective. A beam expander may actually be required rather than a beam condenser in these cases, because the convergent laser beam reduces in width as it approaches the objective. By choosing the powers of the beam condenser/expander lenses appropriately, and accounting for the distance from the beam expander/condenser to the objective, the width of each laser beam at the objective can be matched to the width required to achieve the chosen depth of field. By using a focal plane short of the objective's back focus, the laser beam widths at the beam expander/condensers and at the laser-beam-combining dichroic optics, will be wider, and will have lower power densities, reducing the need for high power-density coatings and optics.

We next turn to an exemplification of the concept of imaging a single strand of DNA. The fluorescent limit that the instrument would have to detect is the number of

photons emitted from a single strand of fluorescently labeled cDNA probe. An excited fluorophore typically emits a photon in less than 10^{-9} seconds. Consequently, 1000 photons (or more) can be emitted from a single fluorophore in a 10-microsecond collection time, assuming the laser saturates the fluorophore. A 10-microsecond collection time allows acquisition of a 2000x2000, 10-micron pixel array in 40 seconds. CDNA's are typically 1 k bases (1000 bases) in length, and the fluorophore is incorporated into one of the four bases. Incorporation rates are typically 25%. Assuming 1/4th of the cDNA is the fluor-tagged base, then the typical number of fluors per cDNA is 0.25* 1000/4 = 62. The optical efficiency of a 0.3NA system is 0.0224, and the QE of the PMT is 0.15. To calculate the number of photons collected from a single strand of cDNA, we multiply 62 floors * 1000 photons per fluor per 10 microsecond time interval * 0.0224 photons collected by the optics * 0.15 photons amplified by the PMT. The result is that 208 amplified photons are collected in a pixel from a single cDNA strand. This number of photons is easily detected by a PMT. To reduce bleaching effects, the laser power could be reduced by as much as a factor of forty, yielding a photon count of 5, and a single cDNA strand could still be detected reliably. If the PMT gain was adjusted to bring the photon signal above the electronics noise, Schott noise would limit the signal to noise of the system to 2, (5/sqrt(5)), which is enough to reliably identify one strand of cDNA vs. two or more. The ideal laser level would be at ~ 1/4 of the saturation level of the fluorophore, where the signal is strong, the fluorophore response is linear, and bleaching is less likely.

The excitation and emission spectra for fluorescent dyes cover a broad range of the visible spectrum, and when several are used simultaneously, there is inevitably some overlap. Hence, the need for some form of crossover correction. For optimal detection of each fluorophore, a mixed sample can be scanned sequentially. The best color for exciting one of the dyes is used in combination with the best filter for collecting only emission from this dye, then the best combination for the next dye is used and so on until all dyes have been detected. This greatly improves quantitative data acquisition, at the expense of scanning speed. The scanner described above can take data either sequentially or simultaneously, under computer control.

However, if one dye is stimulated by illumination intended for another, and if its emission spectrum overlaps the collection range, this dye will contribute some signal to the wrong channel, an effect known as crossover or bleed-through. If the amount of bleed-through between channels is properly characterized for a particular set of dyes, and a particular set of imaging parameters, a crossover compensation algorithm can be used to remove it from the data. Percentage crossover values are tabulated for each dye, then a pixel by pixel subtraction is carried out between the different images to eliminate that portion of the signal which is known to have come from the wrong channel.

FIGS. 11-14 illustrate opto-mechanical features that allow the scanner to operate in a dark-field mode, eliminating laser-light back scatter from lens surfaces in the optical path and from the slide surfaces. Dark field operation reduces the laser-blocking and minimal fluorescence requirements on the emissions filters in front of the PMT, reducing background in the image, and improving the sensitivity of the instrument. The opto-mechanical features also eliminate ghost-images, which can be created by small amounts of laser light reflecting off from the back of a clear slide on to a fluorescent feature on the top of the slide. Because this invention eliminates scattered excitation light from the detector area, it acts as a dark-field instrument, increasing the overall sensitivity of the system.

Ghost images are caused by excitation light 115 from the laser beam reflecting off of the back surface of the slide, and exciting fluorescence from fluors 116 on the top surface of the slide (Fig.11a.) In the scanning configuration, the computer records the position of the point at the first-strike position of the laser beam on the slide 117 (Fig. 11b). The instrument collects fluorescence from this point 117, but also collects fluorescence from the second strike position 116 (Fig. 11b) of the reflected laser beam. The reflected laser beam is weaker than the primary beam and is up to 4% of the primary beam's power, depending on vignetting and the reflective properties of the slide surface. A weaker secondary image, displaced from the original image, results after compilation of all collected data points. The amount of displacement is dependent on the scan angle of the laser beam 24 as the galvanometer mirror 56 scans the beam across the slide (Fig. 11c). The ghost image 116 lies directly on top of the

primary image 118 when the laser beam is orthogonal to the slide (at a 0-degree scan angle), and in this position the ghost is not visible in the image.

The collected fluorescence light can be imaged to a point, and the visible part of the ghost images can be rejected with a confocal point aperture. However, there are at least two problems with this technique. First, at and near the 0-degree scan angle, the ghost image point 116 lies partially or fully on top of the primary image point 118 and cannot be rejected by the confocal aperture. Consequently, the collected fluorescence intensity varies with scan angle, adding an error to the quantitation of the fluorescence. Second, a pinhole aperture gives a curved confocal optical section, which is undesirable in this application, which requires a deep depth of field over a flat slide surface, to accommodate small changes in axial focus with automated slide insertion into the instrument.

In a preferred embodiment, the optical system can be modified to tilt the incoming laser beam in the direction orthogonal to the galvanometer mirror scan of the laser beam. This displaces the ghost images in a direction orthogonal to their original appearance (Figs. 11d and 11e). The ghost image point 116 displaced in this direction can be eliminated completely with a straight aperture on one side of the PMT, without creating a curved confocal section. Since the ghost image point 116 is displaced for all scan angles, it can be rejected by the aperture at all scan angles, eliminating the variation in fluorescence collection with scan angle.

Alternatively, an opaque slide can be used in place of a clear slide, eliminating the secondary laser-beam reflection, and any consequent ghost images. A clear slide can also be turned over, so the DNA is imaged through the glass slide. This eliminates the first laser reflection. However, another laser reflection can still cause weaker ghost images in this configuration. The laser beam is reflected twice, once off of the bottom of the slide, once off of the top, and then strikes the displaced fluorescent object. These ghost images would have a maximum intensity of 0.16% of the original fluorescent object, on a typical clear slide.

Although the optics may have anti-reflective coatings, laser beam reflections from the surface of the optics can still be 0.5% or more. Laser back-scatter from a clear slide can be 4% or more. Often times the fluorescence signal can be many

orders of magnitude (>10^6) less than the exciting laser power. Laser-beam back reflections 124 from the lens surfaces can be many orders of magnitude brighter than the fluorescence that is being collected, putting extreme requirements on the PMT emission filters 127 for ultra-low fluorescence properties, and extremely effective laser-light rejection (Fig. 12a). However, the optical path can be modified to cause back-scattered laser light to be apertured in the optical system, and miss the PMT aperture altogether, creating effective dark-field conditions.

The back reflection from the slide surface misses the PMT 123 at larger scan angles, and only reaches the PMT at scan angles near 0 degrees. Consequently the image may have a band of increased background down its center, if the emission filters fluoresce at all, or do not sufficiently reject the laser light.

The laser beam in a preferred embodiment is smaller than the collected fluorescence beam. It can be aligned off-center 126a a small amount, negligibly affecting the quality of the laser-spot from using the lenses slightly off-axis. However, the off-axis alignment also scatters the back-scattered laser light off-axis 125. The off-axis back-scatter is then apertured at various places in the optical system, and misses the PMT altogether (Fig. 2b). This off-axis alignment can be made in any direction, and can be moved in a direction that enhances the additional modifications made below (Fig.13a).

In a preferred embodiment, the optical system may be tilted on the scanning y-stage to tilt the laser beam in a direction orthogonal to the galvanometer mirror scan (Fig.13a). This displaces the ghost image 116 orthogonal to the mirror scan of the laser beam (Fig.13b). Fluorescence from the ghost image is imaged at the PMT 122 and removed with a straight-edge aperture 128 that is as at least as long as the PMT opening (Fig.13a). The tilt of the optical system is sufficient to displace the ghost image point enough that it can be apertured without causing a confocal affect. A curved optical section is not created because the aperture is parallel to the scanning-mirror direction. Figures 13a and b show the optical path of the primary image and the ghost image.

The tilted optical system not only rejects ghost images, but also causes the laser back-scatter 132, 133 from the slide to be deflected at a large angle (Figs. 14a

and b). The off-center alignment is made in the appropriate direction to increase the deflection angle even more. If the deflection angle is large enough, the back-scatter from the slide will be apertured at a point in the optical system, and will not reach the PMT. If the angle is not quite large enough, an aperture can be added to a lens or mirror in the optical system to reject the back-scatter (Fig. 14a), with a small loss of fluorescence-collection numerical aperture. The y-stage is moved the same way as in the original configuration, to scan the optics across the length of the slide.

It is to be understood that the above description is intended to be illustrative and not restrictive. Many embodiments will be apparent to those of skill in the art upon reading the above description. The scope of the invention should, therefore, be determined not with reference to the above description, but should instead be determined with reference to the appended claims, along with the full scope of equivalents to which such claims are entitled. The disclosures of all articles and references, including patent applications and publications, are incorporated herein by reference for all purposes.

CLAIMS

What is claimed is:

1. A laser-based optical scanning system for imaging one or more samples on one or more substrates, said optical scanning system comprising:

a laser delivery system comprising at least one laser light source for generating an excitation beam, said beam being generally directed to said sample on said substrate;

a laser-based scanning subsystem and detection subsystem with means for scanning said excitation beam along one or more axes of said substrate and detecting emissions given off by said sample that has been excited by said excitation beam; and

an automated stage with means for transporting one or more of said substrates past said laser beam.

2. A laser-based optical scanning system as recited in claim 1, wherein said laser delivery system includes at least one mirror.

3. A laser-based optical scanning system as recited in claim 2, wherein said mirror is adjustable.

4. A laser-based optical scanning system as recited in claim 2, wherein said mirror is selected from the group consisting of a dichroic mirror and a corner mirror.

5. A laser-based optical scanning system as recited in claim 2, wherein said laser delivery system includes at least one shutter.

6. A laser-based optical scanning system as recited in claim 5, including a controller for controlling at least one the following components selected from the group of components consisting of a laser, mirror and shutter.

7. A laser-based optical scanning system as recited in claim 6, wherein said controller is controlled by software.

8. A laser-based optical scanning system as recited in claim 1, including a plate having an aperture and a transmission mirror, wherein said transmission mirror is used to conduct said laser beam through said aperture.

9. A laser-based optical scanning system as recited in claim 8, wherein said transmission mirror is a dichroic mirror.

10. A laser-based optical scanning system as recited in claim 1, wherein said scanning subsystem includes said substrate is generally rectangular and has a comparatively shorter X-axis and a comparatively longer Y-axis.

11. A laser-based optical scanning system as recited in claim 1, wherein said laser-based scanning system includes a galvanometer mirror.

12. A laser-based optical scanning system as recited in claim 1, wherein said laser-based scanning system includes an objective scanner.

13. A laser-based optical scanning system as recited in claim 1, wherein said laser beam is focused, and the movement of said stage effectuates the scanning of said laser beam across said scanning mirror.

14. A laser-based optical scanning system as recited in claim 4, wherein said objective scanner includes a pentaprism.

15. A laser-based optical scanning system as recited in claim 1, wherein said stage is positioned at or in close proximity to the focal point of said laser beam.

16. A laser-based optical scanning system as recited in claim 1, wherein said laser beam originates above said slide and is directed by a mirror to said slide where it excites said sample positioned on said slide.

17. A laser-based optical scanning system as recited in claim 1, wherein an aperture of a component positioned between said laser-delivery system and said

substrate separates a majority of laser illumination scatter and prohibits the majority of scatter from continuing to the components of said system located beyond the component having the aperture.

18. A laser-based optical scanning system as recited in claim 1, wherein said system includes at least one excitation dichroic, and wherein the fluorescence generated by said sample on said substrate slide that is excited by said laser beam is collected along the same optical path as said laser beam by passing through the said excitation dichroic to a detection system.

19. A laser-based optical scanning system for imaging one or more samples on one or more substrates, said system comprising:

at least two base plates, including an upper base plate with an aperture and a lower base plate positioned below the upper base plate;

a laser delivery system positioned above the upper plate, the laser delivery system being used to position a laser beam;

a laser-based scanning and detection system positioned below the upper plate;

at least one dichroic mirror to direct a laser beam to a slide;

a galvanometer mirror to scan the laser beam along at least one direction; and

an automated stage for transporting one or more substrates, said stage positioned above the lower base plate.

20. A laser-based optical scanning system as recited in claim 19, wherein said stage is positioned an appropriate distance from said laser-delivery system so that said substrate on said stage is positioned at the focal point of the laser beam.

21. A laser-based optical scanning system as recited in claim 19, wherein the laser beam originates above the upper plate, travels through the aperture, and is directed by a mirror to the slide where it excites a sample positioned on the slide.

22. A laser-based optical scanning system as recited in claim 19, wherein the aperture of the upper plate separates a majority of laser illumination scatter and prohibits the majority of scatter from continuing to the components of the system located below the upper plate.

23. A laser-based optical scanning system as recited in claim 18, wherein the fluorescence generated by a sample on the slide that is excited by the laser beam is collected along the same optical path as the laser beam but passes through an excitation dichroic to a detection system.

24. A laser-based optical scanning system as recited in claim 1 wherein said laser-based scanning system includes a galvanometer driven mirror.

25. A laser-based optical scanning system as recited in claim 25 wherein said mirror is elongated in the plane of the long axis of said substrate.

26. A laser-based optical scanning system as recited in claim 1 wherein said laser-based scanner includes an objective scanner.

27. A laser-based optical scanning system as recited in claim 26, wherein said laser-based scanner includes a combination of an objective scanner and said galvanometer-driven mirror.

28. A laser-based optical scanning system as recited in claim 27 wherein said objective scanner scans a focused laser beam across said elongated galvanometer mirror.

29. A laser-based optical scanning system as recited in claim 1, wherein said laser-based scanner includes a pentaprism or pentaprism mirror set.

30. A laser-based optical scanning system as recited in claim 27, wherein said objective scanner includes an objective lens and pentaprism mounted together on a stage with means to effect scanning along one or more axes of said substrate.

31. A laser-based optical scanning system as recited in claim 27, wherein said objective scanner is mounted on cross roller positioning stages.

32. A laser-based optical scanning system as recited in claim 27, wherein the stage drive mechanism of said objective scanner contains an anti-backlash ball leadscrew assembly.

33. A laser-based optical scanning system as recited in claim 27, wherein said objective scanner is driven by a microstepping motor.

34. A laser-based optical scanning system as recited in claim 27, wherein said objective scanner is driven by a DC servo motor with position feedback mechanism.

35. A laser-based optical scanning system as recited in claim 1, wherein an aperture positioned between the laser-delivery system and the sample separates a majority of laser illumination scatter and prohibits the majority of scatter from continuing to the components of the system located between the aperture and one or more detectors.

36. A laser-based optical scanning system as recited in claim 1, wherein the system includes one or more excitation dichroics, and wherein the fluorescence generated by a sample on the substrate is collected along the same optical path as the laser beam, by passing through one or more excitation dichroics to a detection system.

37. A laser-based optical scanning system as recited in claim 37, wherein said system includes one or more Rugate filters in place of one or more excitation dichroics.

38. A laser-based optical scanning system as recited in claim 25, wherein the stage is positioned an appropriate distance from the laser delivery system so that the desired depth of field is surpassed and hence the laser spot is prevented from being blurred by the field curve of the scanning mirror.

39. A laser-based optical scanning system as recited in claim 1, wherein oversized optics are used to collect a high NA from the excited fluors in the laser spot.

40. A laser-based optical scanning system as recited in claim 26, wherein an oversized objective is used off-center to minimize the scan mirror size for a given NA.

41. A laser-based optical scanning system as recited in claim 1 wherein said system employs confocal optics.

42. A laser-based optical scanning system as recited in claim 1 wherein a beam expander and focusing lenses are employed to reduce the required emission filter size.

43. A laser-based optical scanning system as recited in claim 1 wherein integrating electronics are used in one or more photomultiplier tube amplifiers.

44. A laser-based optical scanning system as recited in claim 1 wherein logarithmic electronics are used with one or more photomultiplier tube detectors to extend dynamic range.

45. A laser-based optical scanning system as recited in claim 1 wherein silicon diode detectors are used for high signal channels, such as for total DNA quantitation.

46. A laser-based optical scanning system as recited in claim 1, wherein photon counting is utilized for measuring low-light level signals.

47. A laser-based optical scanning system as recited in claim 1, wherein a one or more miniature CCD array detector is used.

48. A laser-based optical scanning system as recited in claim 1, wherein a CCD pixel or cluster of pixels is used as a confocal aperture.

49. A laser-based optical scanning system as recited in claim 1, wherein crossover-correction routines are utilized.

50. A laser-based optical scanning system as recited in claim 1, wherein the width of the laser beam is manipulated to choose the appropriate depth of field to allow for automated sample handling.

51. A laser-based optical scanning system as recited in claim 1, wherein the sample is moved in the short axis to allow scanning of strips of a sample with a format larger than 25mm wide.

52. A laser-based optical scanning system as recited in claim 1, wherein a bar code reader is used to track the identity of samples.

53. A laser-based optical scanning system for imaging one or more samples on one or more substrates, said system comprising:

at least two base plates, including an upper base plate with an a aperture and a lower base plate positioned below the upper base plate;

a laser delivery system positioned above the upper plate, the laser delivery system being used to position one or more laser beams;

a laser based scanning and detection system positioned below the upper plate;

at least one dichroic mirror to direct a laser beam to a substrate; and

a galvanometer driven mirror to scan the laser beam along at least one direction; and an automated stage for processing multiple samples, the stage positioned above the lower base plate.

54. A laser-based optical scanning system as recited in claim 53, wherein the aperture of the upper plate separates a majority of laser illumination scatter and prohibits the majority of scatter from continuing to the components of the system located below.

55. A laser-based optical scanning system as recited in claim 1, wherein scanning is achieved by moving the objective in two dimensions.

56. A laser-based optical scanning system as recited in claim 55, wherein a small, lightweight, high NA objective is used with a pentaprism to achieve maximal scan speed and collection efficiency.

57. A laser-based optical scanning system as recited in claim 55, wherein two pentaprisms are used with a two-way stage scan mechanism to stabilize the scanning and minimize vibration effects.

58. A laser-based optical scanning system as recited in claim 55, wherein the two-way stage scan mechanism utilizes linear motors.

59. A laser-based optical scanning system as recited in claim 55, wherein the objective lens is achromatic.

60. A laser-based optical scanning system as recited in claim 55, wherein the objective lens is monochromatic to minimize focal length, weight and size while maximizing the NA.

61. A laser-based optical scanning system as recited in claim 60, wherein the collected beam size is minimized to reduce the size and weight of the pentaprisms.

62. A laser-based optical scanning system as recited in claim 55, wherein a beam condenser is used to manipulate the width of the laser beam at the objective, in order to regulate the depth of field.

63. A laser-based optical scanning system as recited in claim 55, wherein a beam condenser is used to manipulate the width of the laser beam at the objective, in order to regulate the spot size on the specimen and hence the spatial resolution.

64. A laser-based optical scanning system as recited in claim 55 wherein beam expanders are used with each laser to correct for chromatic errors in the objective and cause the focal planes for all lasers to be coincident.

65. A laser-based optical scanning system as recited in claim 55 wherein one axis is scanned in multiple passes down the other axis.

66. A laser-based optical scanning system as recited in claim 55 wherein one of the scan mechanisms is also utilized to automate sample loading.

67. A laser-based optical scanning system as recited in claim 55 wherein a sample with a different format or substrate than a microscope slide is imaged.

68. A laser-based optical scanning system as recited in claim 55 wherein confocal optics are utilized.

69. A laser-based optical scanning system as recited in claim 55 wherein a beam expander and focusing lenses are used to minimize detection filter size.

70. A laser-based optical scanning system as recited in claim 1 wherein means are used to adjust the angle of said excitation beam relative to the scan of said sample by a galvanometer mirror.

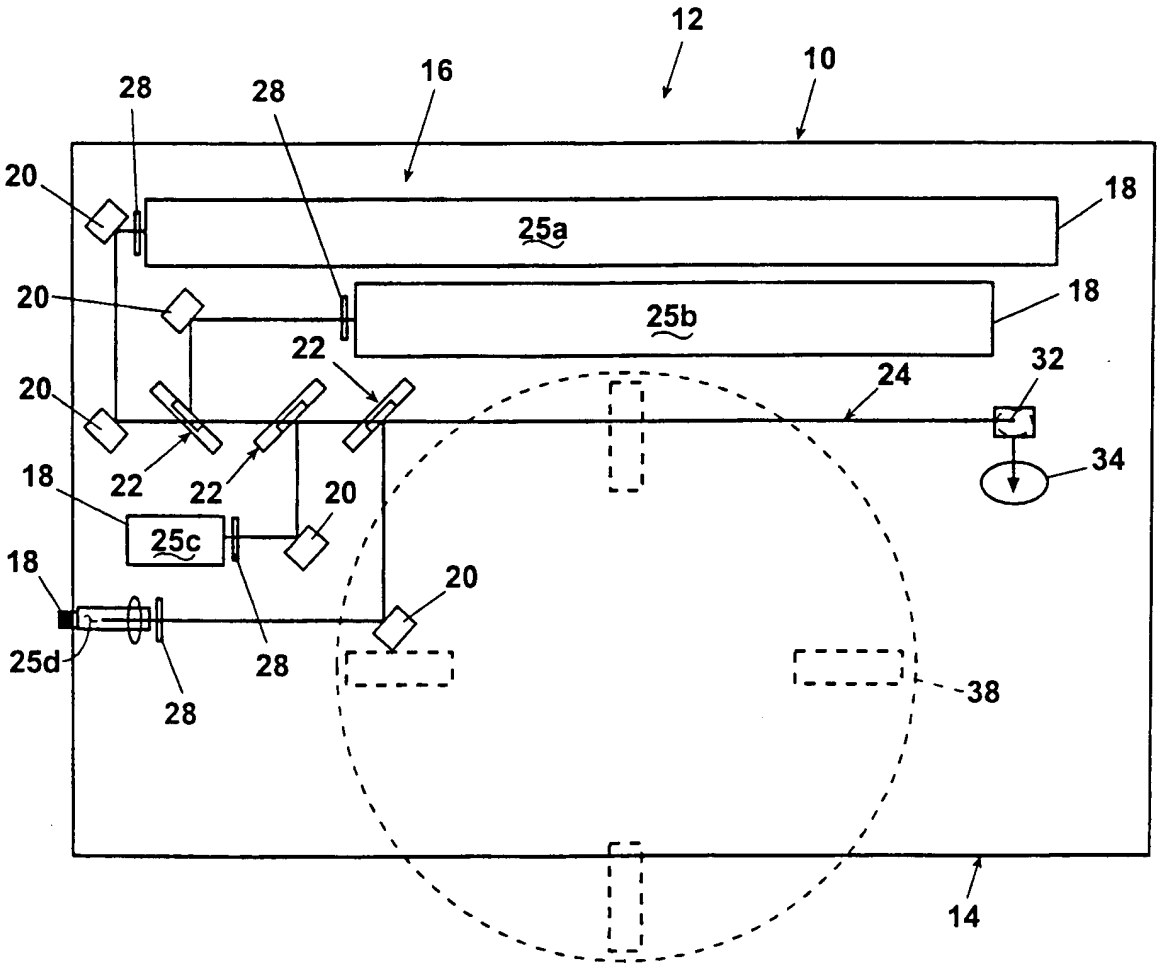
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Fig. 1

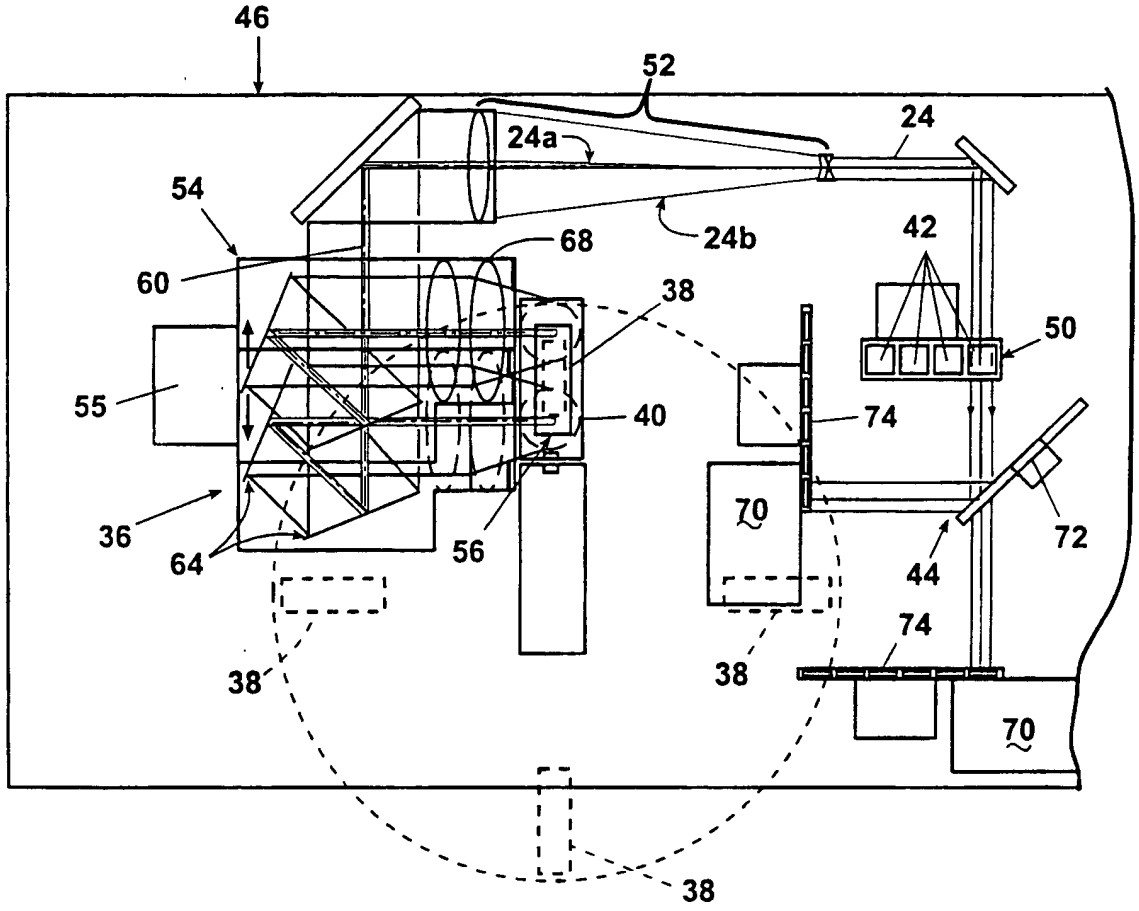
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Fig. 2a

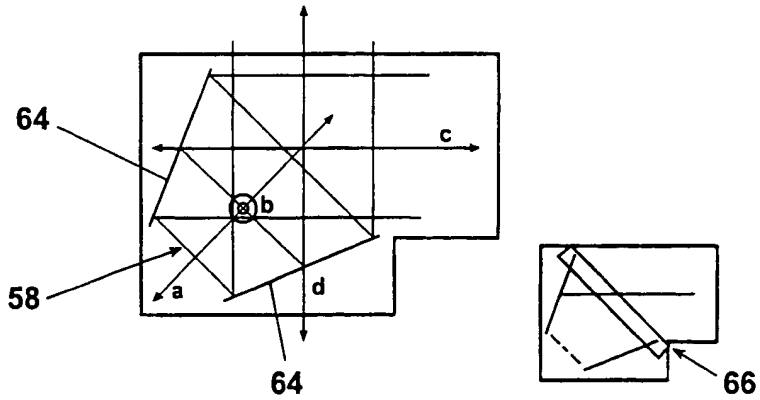
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Fig. 2b

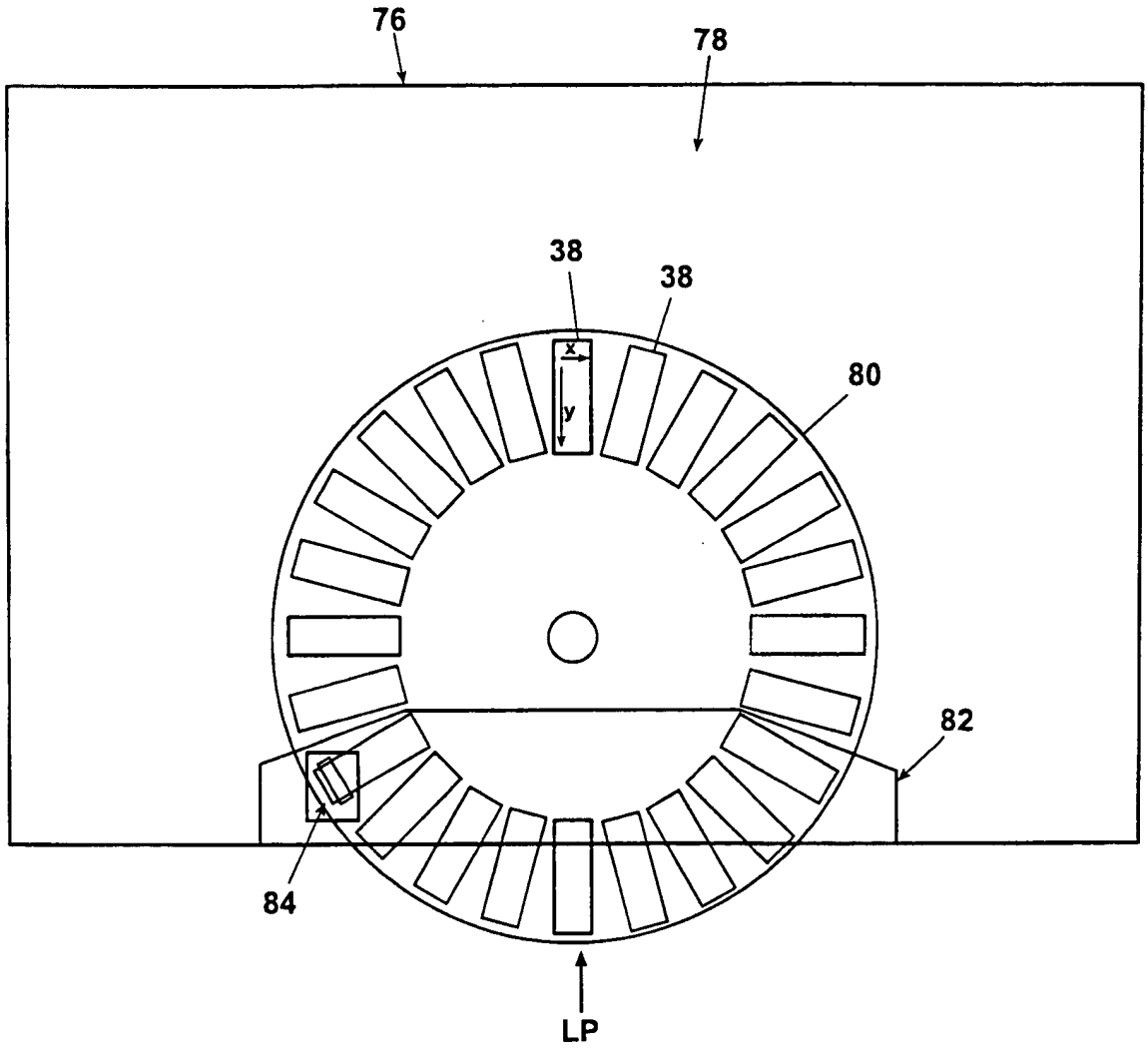


Fig. 3

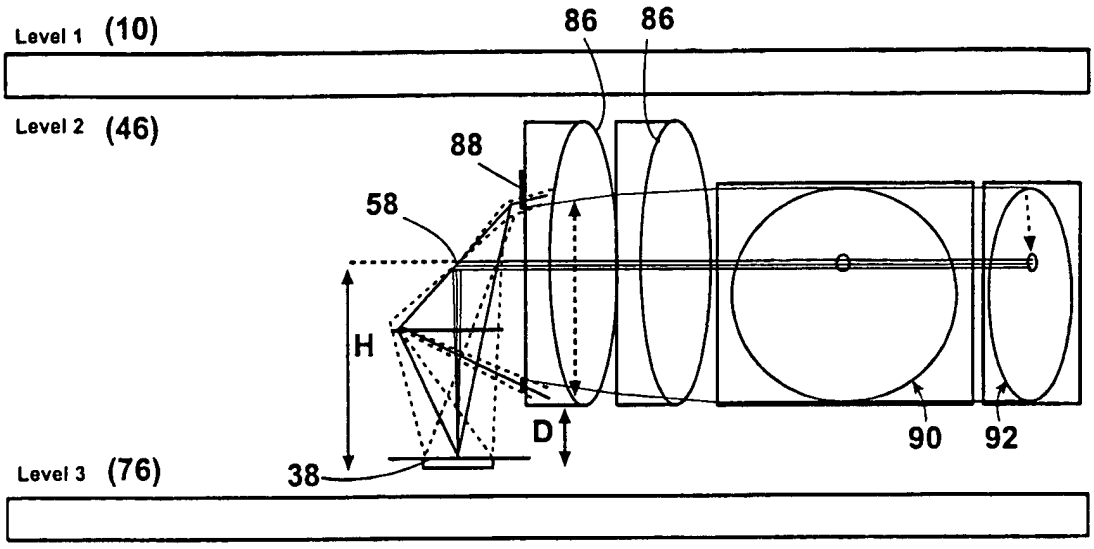
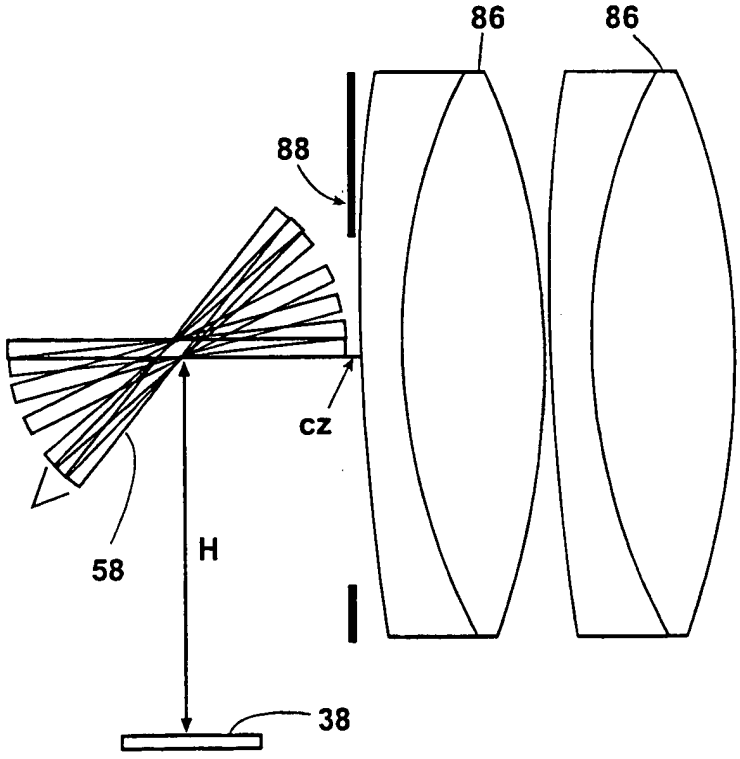

Level 1 (10)
Level 2 (46)
Level 3 (76)
86
86
88
58
H
D
38
90
92

Fig. 4a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88
cz
58
H
38

Fig. 4b

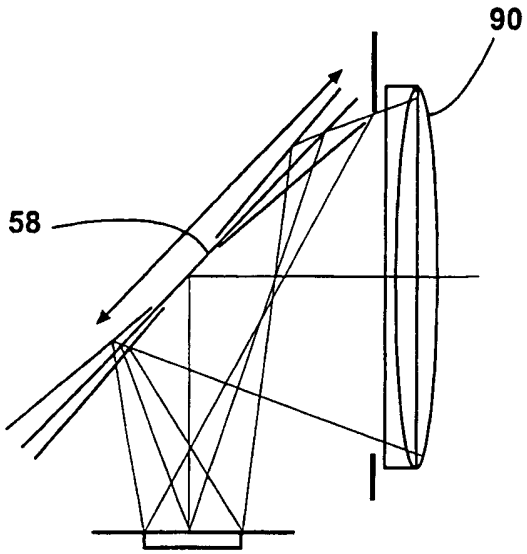


Fig. 5a

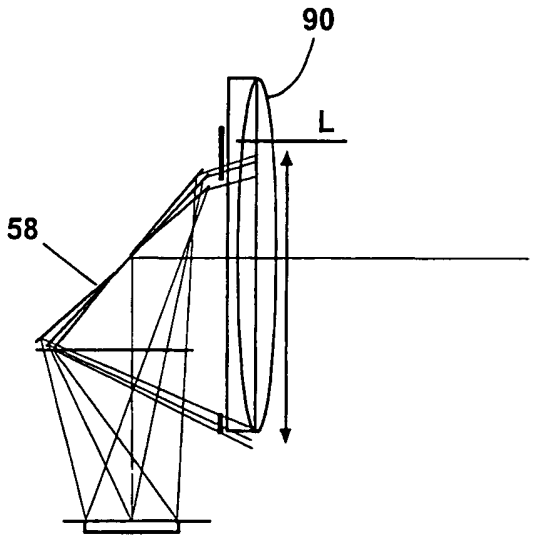


Fig. 5b

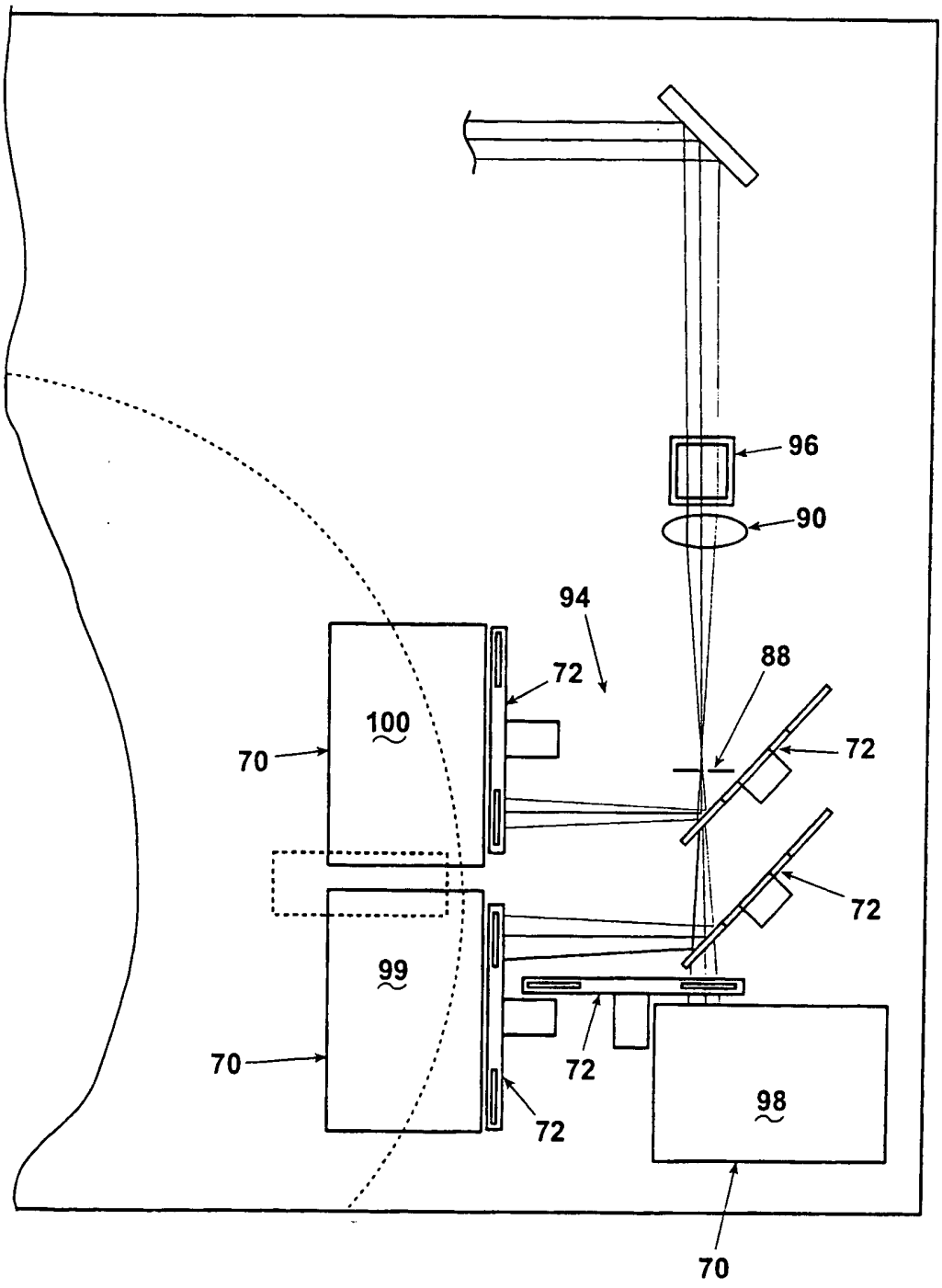


Fig. 6

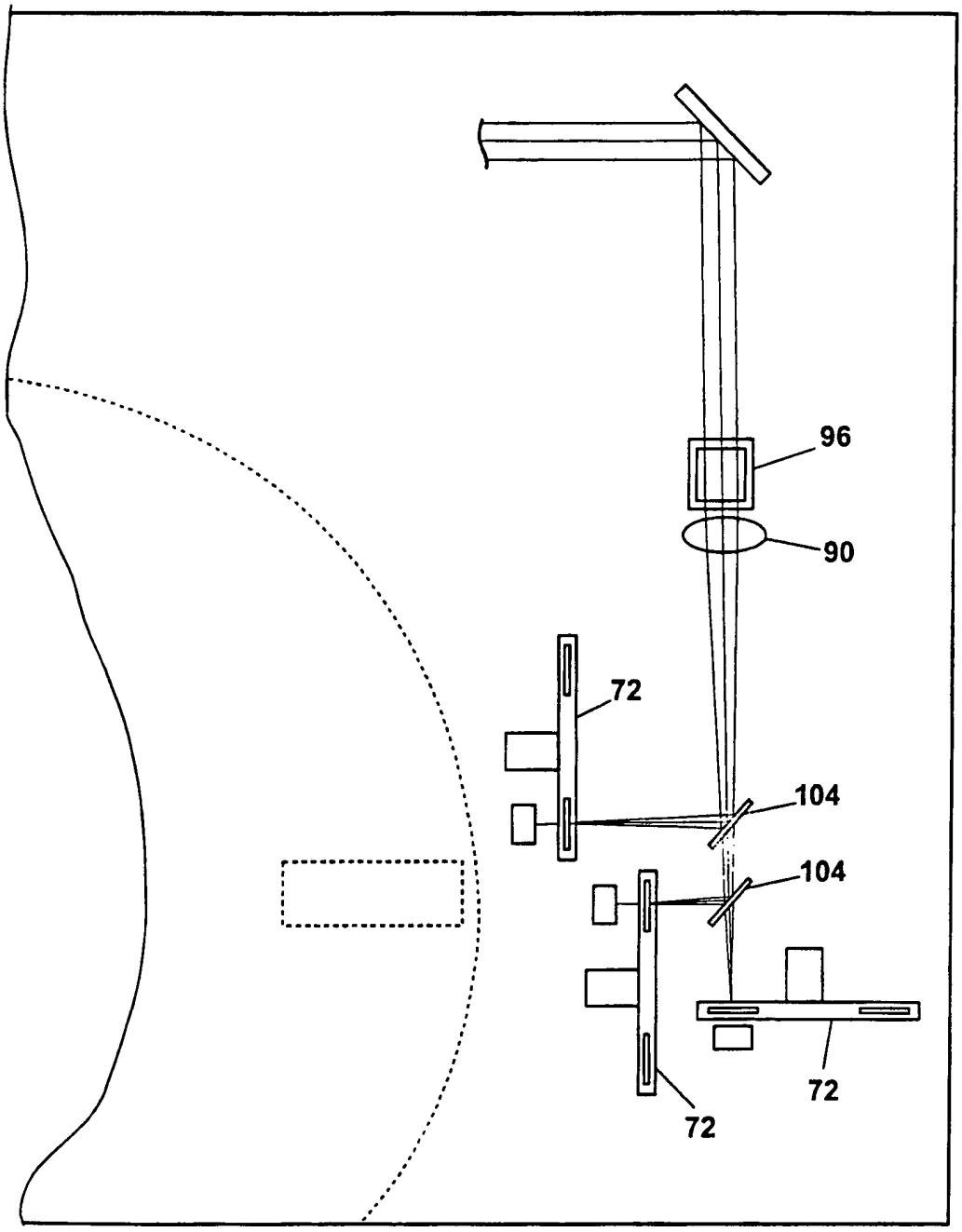
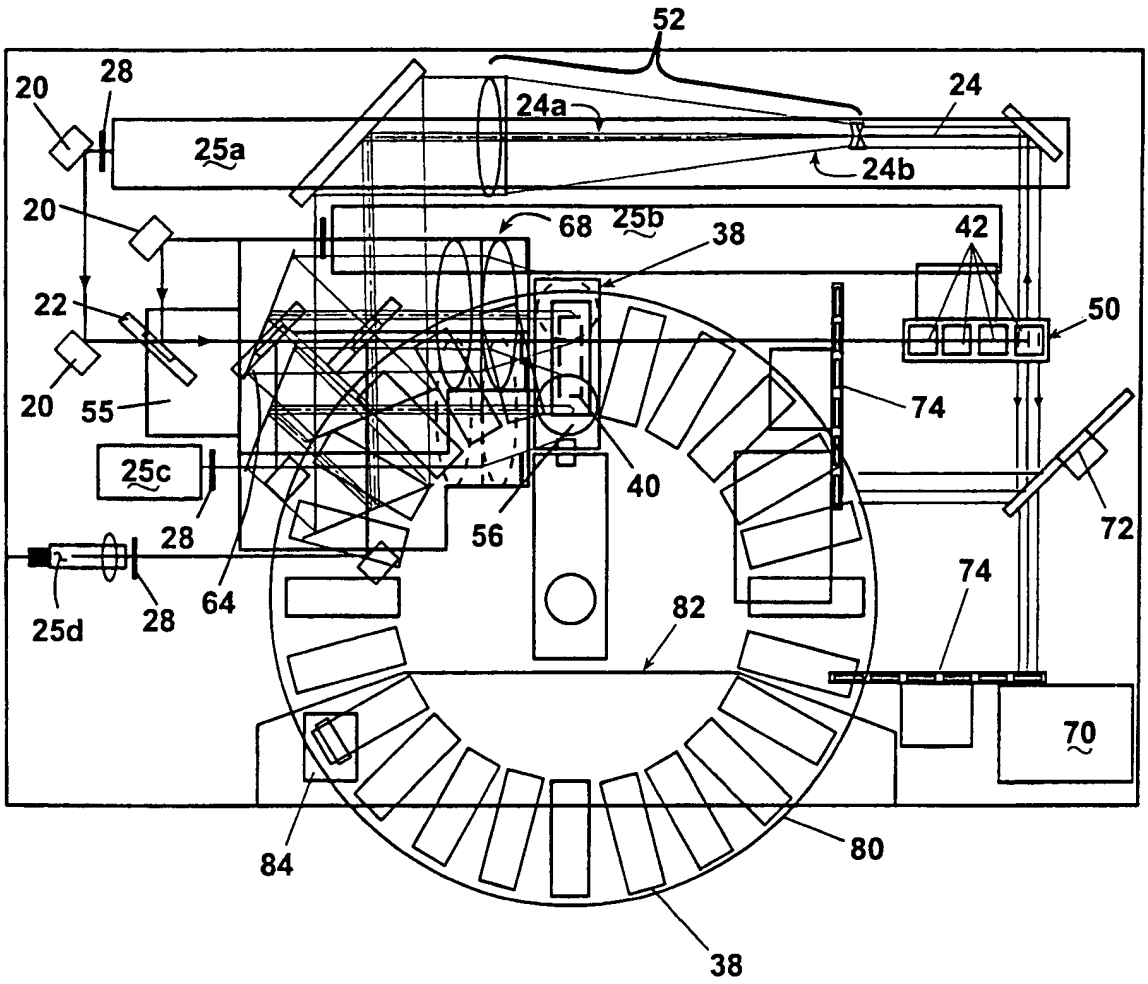


Fig. 7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25a
24b
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25b
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25c
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25d
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84
80
38

Fig. 8

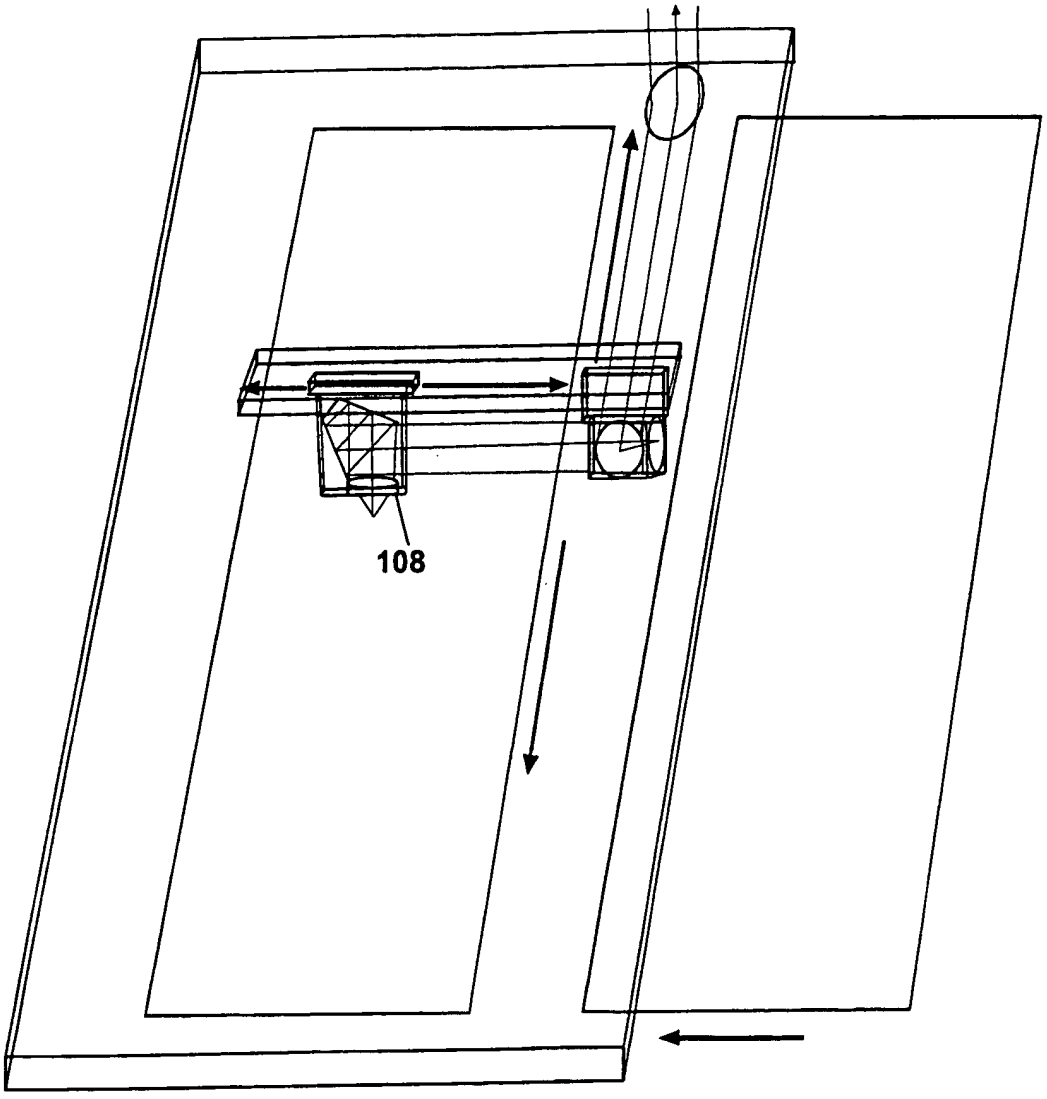

Fig. 9

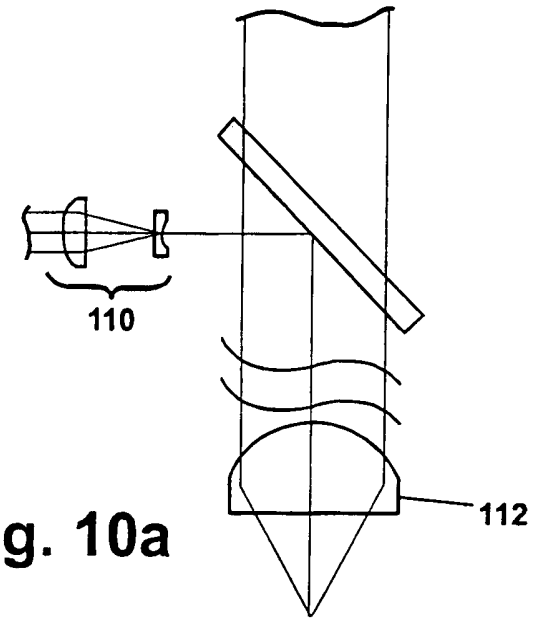

Fig. 10a

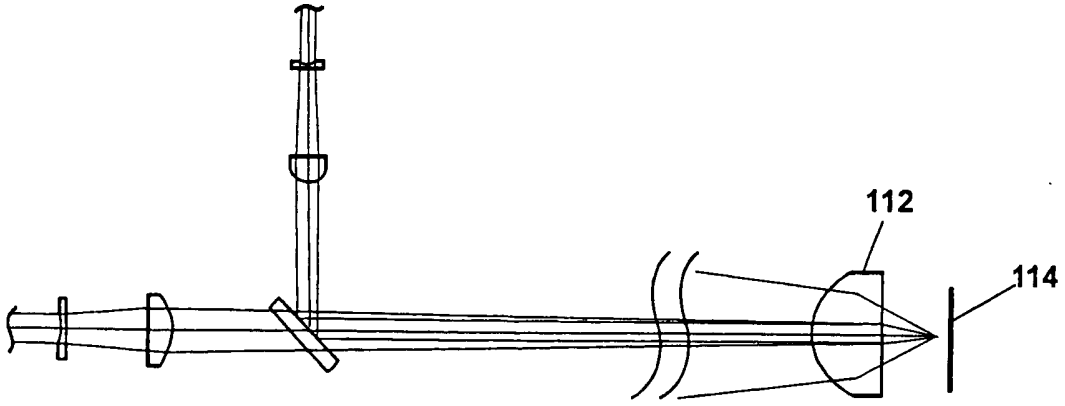

Fig. 10b

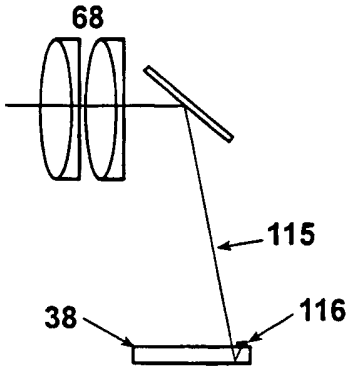

Fig. 11a

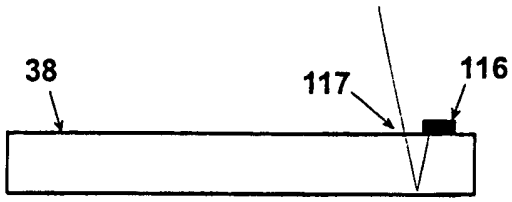

Fig. 11b

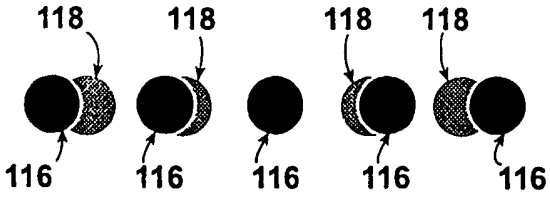

Fig. 11c

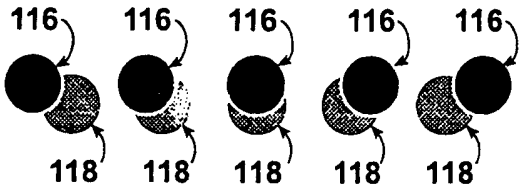

Fig. 11d

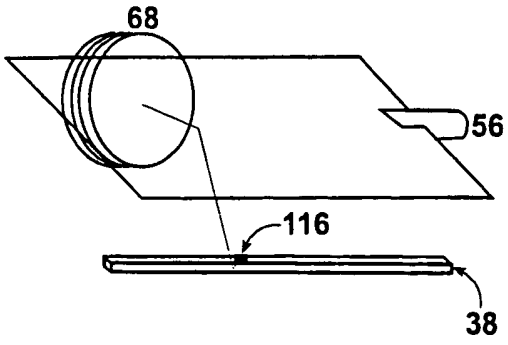

Fig. 11e

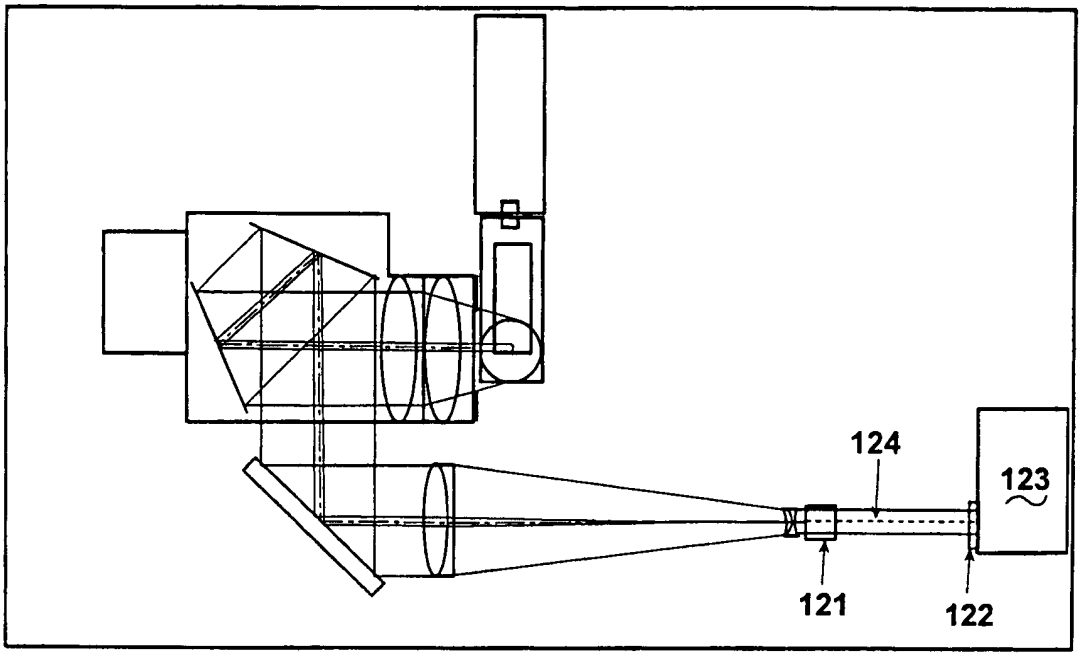

Fig. 12a

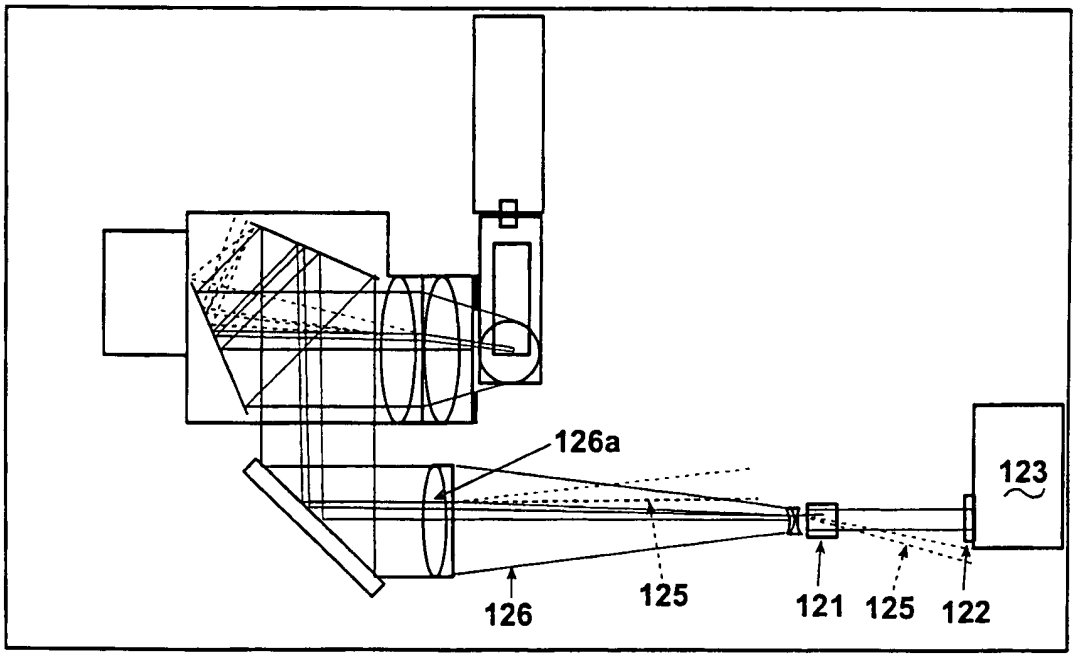

Fig. 12b

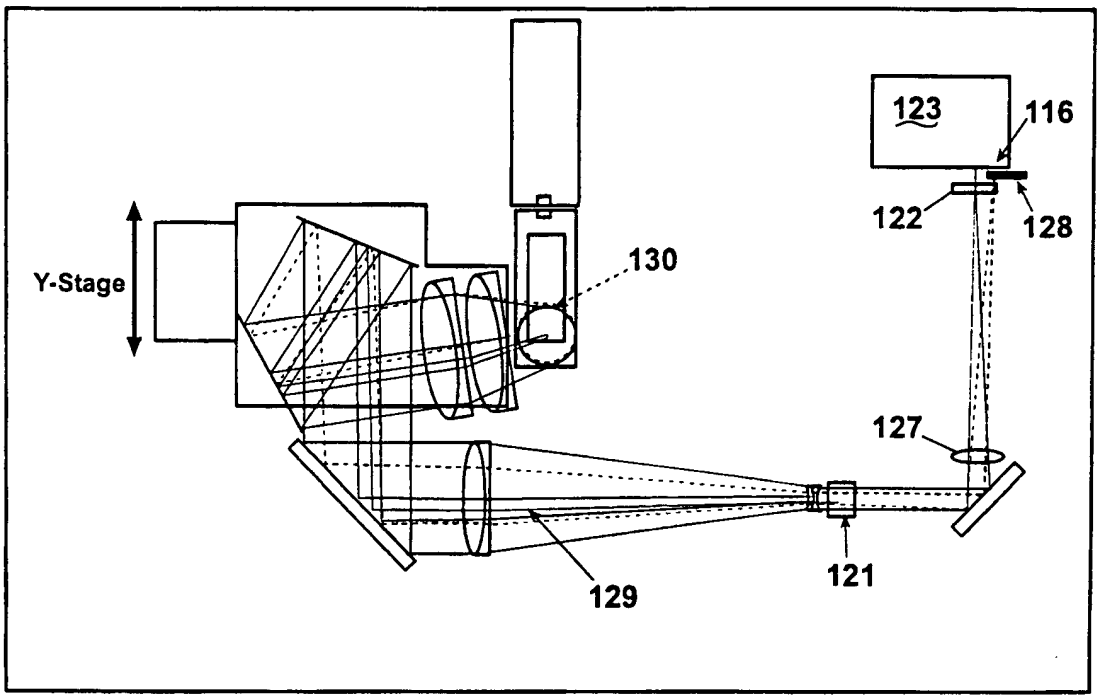

Fig. 13a

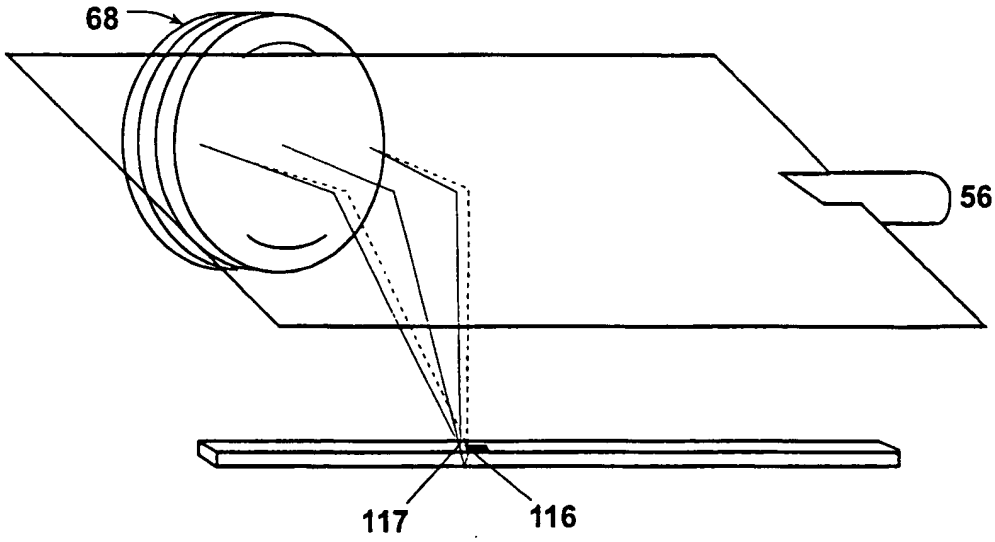

Fig. 13b

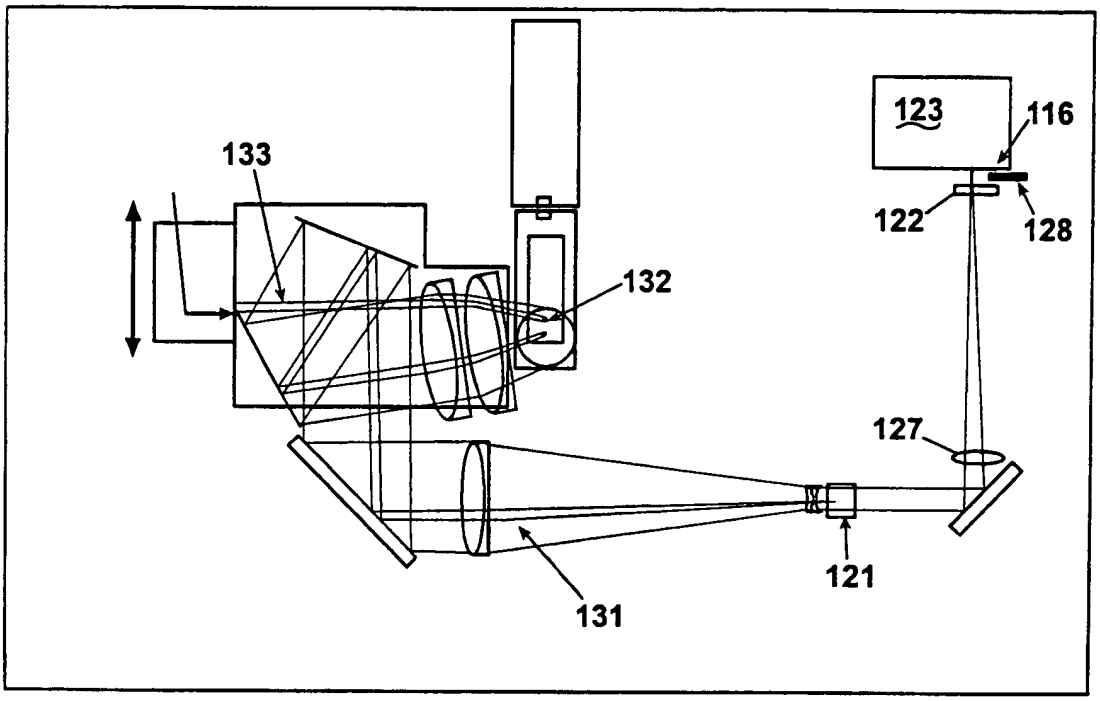

Fig. 14a

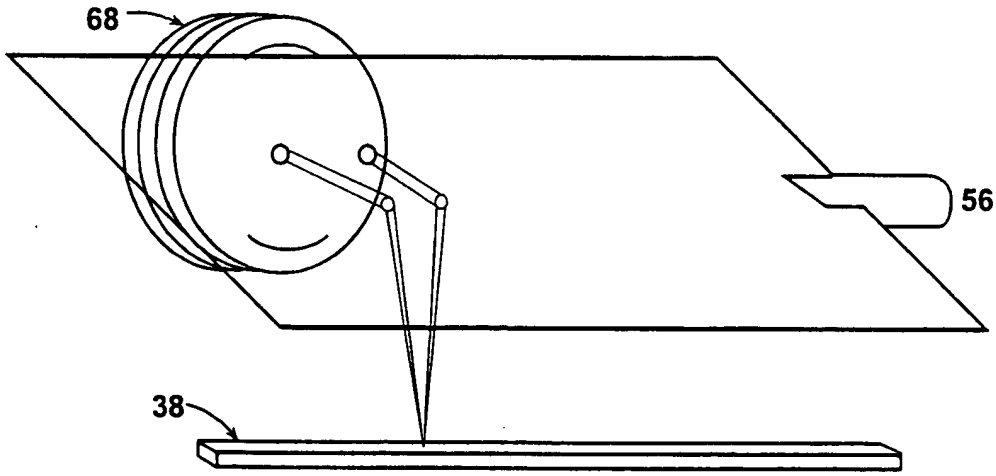

Fig. 14b

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/12220

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :G02B 26/08

US CL : 359/212-215, 391, 394

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 359/204, 211-215, 368, 391-394, 738-739, 833-834

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 5,537,247 A (XIAO) 16 July 1996 (16.07.1996), Figs. 1-6 and the accompanying text.	1-2, 4-13, 15-24, 26-28, 33-36, 39-45, 47-51, 53-54 --- 55, 58-60, 62-70
X	US 5,895,915 A (DeWeerd et al) 20 April 1999 (20.04.1999), Figs. 1, 3-4, 6 and the accompanying text.	1-2, 4-13, 15-24, 26-28, 31-36, 39-45, 47-51, 53-55, 58-60, 62-68 and 70

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

18 JULY 2000

Date of mailing of the international search report

02 AUG 2000

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231

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Authorized officer

JAMES PHAN

Telephone No. (703) 308-4810

Form PCT/ISA/210 (second sheet) (July 1998) ★

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/12220

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 25 AND 37-38
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

THEY ARE DEPENDENT ON THEMSELVES.

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.